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Growth and yield parameters, water relations, and photosynthesis of soybean plants infected with Phialophora gregata

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Growth and yield parameters, water relations, and photosynthesis of soybean plants infected with *Phialophora gregata*

by

Phyllis Maraulja Higley

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INTRODUCTION

Brown stem rot (BSR), caused by Phialophora gregata (Allington and Chamberlain) Gams, is a vascular disease of soybean [Glycine max (L.) Merr.] and adzuki bean. BSR of soybean has been reported in the US, Canada, Mexico, and Egypt (Sinclair and Shurtleff 1975), and BSR of adzuki bean has been reported in Japan (Kobayashi et al. 1983). First isolated in Central Illinois in 1944 and 1945 (Allington 1946), the causal agent of BSR was initially called Cephalosporium gregatum (Allington and Chamberlain 1948). In 1971, Gams placed the pathogen in the genus Phialophora, and species name was changed consequently to gregata (Gams 1971).

In general, the primary physiological effect of vascular diseases is the induction of water stress in the host plant (Talboys 1968). Disease-caused water stress may occur through occlusion of the vascular system resulting in a consequent interruption of the transpiration stream or by excessive loss of water from aerial plant parts (Talboys 1968). Although much is known about the physiological effects of vascular diseases in plants, little is known of the physiological responses of soybean to BSR. Symptoms produced by BSR include vascular browning and interveinal chlorosis and necrosis of leaves (Allington and Chamberlain 1948). Chamberlain and McAlister (1954) reported a reduction in stem conductance proportional to the severity of internal stem browning. Furthermore, a water extract made from BSR-diseased soybean stems has also been shown to reduce stem conductance (Chamberlain 1961). Reductions in stem conductance could induce water

deficiency in the plant. However, it has been suggested that wilting is not due solely to occlusion of xylem vessels, but that one or more metabolites may impose water stress by other means (Gray and Chamberlain 1975). Metabolites produced by P. gregata also have been implicated in inhibition of photosynthetic electron transport (Reeder et al. 1986). Consequently, photosynthesis effects, as well as water stress effects, may be involved in BSR disease.

In fields where P. gregata occurs, BSR has been reported to cause yield losses of 5-56% (Dunleavy and Weber 1967, Gray 1972, Gray and Sinclair 1973, Kennedy and Lambert 1981, Mengistu and Grau 1987, Mengistu et al. 1987, Weber et al. 1966). The variation in loss estimates may have been caused by differences in inoculum source, cultivars grown, cropping history, and environment. Yield data indicate that BSR reduced soybean yield primarily by reducing seed number and secondarily by reducing seed weight (Dunleavy and Weber 1967, Mengistu et al. 1986, Weber et al. 1966), possibly through premature ripening (Chamberlain and McAlister 1954). However, physiological responses of the soybean plant to BSR disease have not been studied in association with growth and yield parameters.

Rotation schemes of three years out of soybean provide good control of BSR (Dunleavy and Weber 1967). However, current cropping practices, which allow only one year out of soybean, do not control BSR (Dunleavy and Weber 1967). Therefore, the use of resistant cultivars is the most efficient control strategy for the disease (Tachibana 1986). However, the nature of resistance has not been well

characterized. In fact, it is unclear whether resistance is expressed in the aerial (Gray and Chamberlain 1975, Kunkel and Dunleavy 1965) or root (Phillips 1971) regions of soybean. Resistance commonly reduces disease severity, although disease incidence may not decrease (Mengistu et al. 1986, Tachibana and Card 1979). Additionally, two resistant plant introductions, PI 84.946-2 and PI 86.150, were shown to resist the wilting effect of an extract made from diseased soybean stems (Gray and Chamberlain 1975). Therefore, resistance to pathogen colonization as well as to activity of pathogen metabolites may operate.

A thorough study of BSR effects on plant growth and yield parameters, water relations, and photosynthesis has not been conducted. Additionally, a detailed comparison of these characteristics has not been made between resistant and susceptible cultivars. The overall objective of this study was to determine the effects of brown stem rot on certain parameters of soybean growth and physiology. Specifically, the objectives were to: 1) assess the impact of BSR on stomatal conductance, transpiration, leaf water potential, and stem conductance of soybean, 2) determine whether BSR decreases photosynthesis of soybean, 3) characterize the growth and yield development of soybean infected with P. gregata, and 4) compare each of the above mentioned characters in a resistant and a susceptible cultivar to provide preliminary insight into the mechanism of resistance.

Explanation of Dissertation Format

This dissertation consists of a general introduction, literature review, two separate manuscripts (sections), a general summary,

literature cited, and acknowledgments. The manuscripts do not contain their own literature cited sections. Rather, all references are cited in the overall literature cited section of the dissertation. The Ph.D. candidate will be senior author on publications derived from the two sections.

LITERATURE REVIEW

Historical Information

Symptomology

Brown stem rot (BSR) of soybean [Glycine max (L.) Merr] is caused by Phialophora gregata (Allington and Chamberlain) Gams. The symptoms of BSR include browning of vascular tissue and pith and interveinal chlorosis and necrosis of leaves. Vascular and pith browning may be observed in midwestern United States beginning in late July to early August. Foliar symptoms, when they occur, are expressed in late August to early September (Allington and Chamberlain 1948). Gray (1972a) observed severe foliar symptoms three weeks prior to plant maturity.

Nomenclature

The initial description of the disease was made by Allington in 1946 from soybean fields affected in 1944 and 1945 in Central Illinois (1946). Allington and Chamberlain (1948) originally called the pathogen Cephalosporium gregatum Allington and Chamberlain, and it was not until 1971 that Gams placed it in Phialophora, and the species name was changed to gregata to agree in gender with the genus name. Much of the literature referenced in this dissertation refers to work done with C. gregatum. I have taken the liberty to refer to the fungus studied in these published works as P. gregata regardless of whether or not the original authors used that taxon.

Host range

P. gregata can infect soybean, mung bean (*Phaseolus aureus* Roxb.) (Allington and Chamberlain 1948), red clover (*Trifolium pratense* L.) (Dunleavy 1967), pinto bean (*Phaseolus vulgaris* L.) (Goth 1966), and adzuki bean (*Phaseolus angularis* Willd. W. F. Wight) (Kobayashi and Ui 1977). Although *P. gregata* has been found to naturally infect soybean, adzuki bean, and red clover in the field, it has not been shown to infect pinto or mung beans unless artificially inoculated into the hypocotyl. Allington and Chamberlain (1948) were unable to reisolate *P. gregata* from artificially inoculated red clover plants. Furthermore, the infected clover found by Dunleavy (1967) occurred in a severely infested soybean field. Besides Dunleavy's report, no further cases of red clover susceptibility to *P. gregata* have been reported. Therefore, *P. gregata* may be a xenoparasite (Luke et al. 1987), rather than a true pathogen, of mung bean, pinto bean, and red clover.

World distribution and agronomic effects

BSR of soybean has been reported in the U.S., Canada, Mexico, and Egypt (Sinclair and Shurtleff 1975). BSR occurs in Japan on adzuki bean, but not soybean, unless artificially inoculated (Kobayashi et al. 1983). In fields where *P. gregata* occurs, BSR has caused yield reductions of 5-56% as compared to fields lacking the pathogen (Dunleavy and Weber 1967, Gray 1972a, Gray and Sinclair 1973, Kennedy and Lambert 1981, Mengistu and Grau 1987, Mengistu et al. 1987, Weber et al 1966). By comparison of soybeans grown in the field on infested

versus noninfested land, Weber et al. (1966) found that soybeans grown on infested land were 2.5 cm taller, matured two days earlier, were 8% less susceptible to lodging, were 0.1 g per 100 seeds lower in seed weight, produced 10.4% fewer seeds, and produced 11% lower yield. They calculated that 94% of the yield loss was due to seed number reduction and 6% was due to seed size reduction. Similarly, Mengistu et al. (1986) found a 17% yield advantage with resistant cultivars over susceptible cultivars, and reported that seed weight was influenced more by the number of seed-bearing pods than by seed size. Furthermore, they determined that resistant cultivars selected on the basis of either stem browning or foliar symptoms showed reduction of both types of symptoms. Mengistu and Grau (1986) and Tachibana (1971) found a height reduction in P. gregata inoculated plants.

Etiology

Pathogen variation

Two strains of P. gregata have been identified (Gray 1971). The Type I, or defoliating, strain causes interveinal chlorosis and necrosis of leaves and extensive browning of the vascular system. The Type II, or nondefoliating, strain does not cause foliar symptoms, and the resultant vascular browning may be less extensive than that caused by the Type I strain. In an evaluation of 35 P. gregata isolates, Gray reported that the Type I strains caused an average of 215 mm of browning while the Type II strains averaged 107 mm of browning. In contrast, Mengistu and Grau (1986) found considerable variation in both

the defoliating and vascular browning potential of Type I strains. Type I strains fell into three groups, causing 80%, 52%, and 46% internal stem browning accompanied, respectively, by 87%, 36%, and 10% foliar symptom severity. The Type II strains caused an average of 50% internal discoloration and no foliar symptoms. Because plant height was reduced by the Type I strains, the calculated average lengths of stem browning for the Type I strains (359 mm) is actually less than for the Type II strains (380 mm). Furthermore, when results of the three Type I strain groups were averaged, the percentage stem browning between Type I (59%) and Type II (50%) was not significantly different.

Pathogen distribution within the host

P. gregata can colonize any of the vegetative plant parts (Gray 1985, Lai and Dunleavy 1969b). However, the pathogen is largely restricted to the xylem vessels until plant maturity when the pathogen can also be found in the pith. Infection of soybean by P. gregata occurs through main and lateral roots three to four weeks after planting (Allington and Chamberlain 1948). It has been suggested that initial colonization by P. gregata as mycelium causes continuous browning of the vascular tissue by nature of its slow, continuous growth (Schneider et al. 1972). With the occurrence of cool temperatures, spores may be produced and quickly distributed through the transpiration stream so that invasion of stem tissues occurs four to five weeks after initial infection (Gray and Sinclair 1973). Rapid distribution of spores may promote discontinuous stem browning.

Schneider et al. (1972) was able to isolate P. gregata from the stem tip two days after a fungal suspension was added to the plant growth medium. Furthermore, colonization by P. gregata preceded stem browning (Gray and Sinclair 1973, Mengistu et al. 1986, Schneider et al. 1972).

Epidemiology

Temperature effects on the pathogen

The numerous studies evaluating the effects of temperature on P. gregata growth and disease development have yielded conflicting results. Allington and Chamberlain (1948) and Hamilton and Boosalis (1955) both reported that optimum temperature range for sporulation on artificial media was about 15-20 C, and that sporulation ceased at temperatures above 29 C. Gray (1985), however, reported a range of 19-25 C, above which he observed no sporulation. Sporulation from naturally infested soybean straw occurred between 7.5 and 30 C and had an optimum range of 15-20 C (Lai and Dunleavy 1969c). Germination occurs at temperatures between 15 C and 30 C (Allington and Chamberlain 1948, Gray 1985). Although Allington and Chamberlain reported 21-25 C as optimum temperatures, germination occurred at a reduced rate under less optimal conditions. Gray found that alternations of 30 and 35 C for four-hour periods, followed by 23 C for 17 hours allowed germination, but not sporulation. Lai and Dunleavy (1969d) reported that germination could occur following successive cycles of freezing and thawing, although germination decreased with each cycle. Mengistu and Grau (1986) found radial growth of mycelium

to be greatest at 20 C, while Allington and Chamberlain reported an optimum of 22-24 C. However, both groups agreed that growth ceases at about 32 C.

Temperature effects on the disease

Disease development and symptom expression vary with temperature. Gray (1985) found that leaf colonization occurred when leaves were incubated 8 h at 27 C, followed by 17 h at 23 C, but not when incubated 7 h at 33 C, followed by 17 h at 23 C. The consensus of many authors (Allington and Chamberlain 1948, Chamberlain and McAlister 1954, Mengistu and Grau 1986, Schneider et al. 1972) is that symptom expression is favored by temperatures of 18-20 C and inhibited at temperatures greater than 27 C. However, there is disagreement regarding expression of specific symptoms. Allington and Chamberlain (1948) and Mengistu and Grau (1986) reported decreased expression of foliar symptoms at high temperatures, while Gray (1974) never reported this effect. There have been several reports of decreased vascular browning with increased temperature (Allington and Chamberlain 1948, Chamberlain and McAlister 1954, Gray 1974, Schneider et al. 1972) and a few reports that temperature does not affect vascular symptom development (Mengistu and Grau 1986, Tachibana 1971).

Survival

Allington and Chamberlain (1948) reported P. gregata to be soil-borne, yet the pathogen was largely associated with organic debris

(Gray 1972b) and diminished when fields were no longer planted to soybeans (Dunleavy and Weber 1967). Therefore, it would be more appropriate to consider P. gregata a soil invader (Garrett 1956). Based on their inability to recover the fungus from overwintered stems, Allington and Chamberlain (1948) concluded that P. gregata did not overwinter in soybean stems. However, subsequent studies indicated that P. gregata survived in the dead stem tissue. By developing a selective medium that controlled the growth of contaminating fungi, Lai (1968) was able to recover P. gregata from naturally infested soybean stems after three months burial in the field or after ten months storage in the laboratory. Sporulation occurred after soybean straw was buried under field conditions for seven months at depths of 8, 15, 23, or 30 cm (Lai and Dunleavy 1969a). Sporulation was induced in a moist chamber from soybean straw that was held on or above the soil surface for up to 10 months. However, the percentage of straw that sporulated decreased with length of time straw was in the field. Sporulation began after two days of incubation in the moist chamber and continued with successive washings for 40 days (Lai and Dunleavy 1969c). About 77% of the total spore number was produced in the first 10 days. Gray (1972b) was able to observe sporulation on soybean stems only until mid-November after which he reported cortical tissues were too severely decomposed. However, P. gregata was recovered from stems collected the following spring by grinding the straw in a Wiley mill and incubating it on water agar amended with tetracycline HCl and streptomycin sulfate. Gray (1972b) concluded that P. gregata

overwintered as mycelium in the woody stem tissues.

Control

Disease Spread

BSR is most serious where susceptible soybeans have been grown continuously, and until 1981, BSR control relied on a four-year rotation that included three years out of soybeans (Allington and Chamberlain 1948). Without rotation, a 47% increase in disease incidence over an eight year period has been documented (Dunleavy 1966). Similarly, Hildebrand (1952) noted spread of disease from a 0.02 acre area to a 0.3 acre area in a two-year period. Survey results indicate that the incidence of BSR in Iowa increased from 79% in 1972 (Dunleavy and Fischer 1973) to 95% in 1977 (Tachibana and Booth 1979). Rotations involving alternate year plantings of maize and soybean were not adequate to control BSR (Dunleavy and Weber 1967).

Resistance

At least three soybean lines have been identified as genetic sources of resistance; Midwest (Kunkel and Dunleavy 1965), PI 84.946-2 (Chamberlain and Bernard 1968), and PI 86.150 (Tachibana and Card 1972). However, it was not until 1981 that BSR 301, the first resistant soybean line having the resistance of PI 84.946-2, was released for commercial use (Tachibana 1986). Since then, three more public cultivars, BSR 302, BSR 201, and BSR 101, have been released in Iowa. Other state and commercial releases are also available.

Because the roots, but not the stems, of Midwest plants were susceptible to disease, resistance was thought to be restricted to the aerial part of the plant (Kunkel and Dunleavy 1965). Additionally, leaves of PI 84.946-2 and PI 86.150 were resistant to an extract made from a P. gregata culture (Gray and Chamberlain 1975), providing further evidence that resistance is located in the aerial plant parts (Gray 1971). However, Phillips (1971) found hypocotyl-inoculated PI 84.946-2 plants were highly susceptible. In an evaluation of the inheritance of BSR resistance, Sebastian and Nickell (1985) concluded that the resistance of L78-4094 (descendant of PI 84.946-2) was due to a single dominant gene, and the resistance of PI 84.946-2 was from two, nonallelic and independently segregating genes.

Physiology of Vascular Diseases

Because BSR is a vascular disease, it is deemed necessary to provide a general discussion of the responses of plants to various vascular diseases. Many studies have been conducted to examine the physiological effects of vascular diseases on plants. Disease-caused water stress may occur through occlusion of the vascular system resulting in interruption of the transpiration stream or excessive loss of water from aerial plant parts (Talboys 1968). Vascular tissue may be occluded by pathogen hyphae or metabolites or by host degradation products or defense reactions. Excessive water loss may occur as a result of interference of stomatal control or altered permeability of cells resulting from disruption of membrane structure and function.

Stem conductance effects

One of the most common effects of vascular disease is a reduction in stem conductance of the host plant. In Verticillium wilt of tomato, caused by Verticillium albo-atrum, stem conductance may be partially reduced by hyphae or by a metabolite of the pathogen (Threlfall 1959). Additional evidence for the role of pathogen metabolites in vascular occlusion is provided by the work of Van Alfen and Turner (1975a,b). In these studies high molecular weight metabolites were isolated from Corynebacterium insidiosum and Ceratocystis ulmi and were found to reduce stem conductance, probably by impeding water transport through pit membranes.

Membrane effects

The occurrence of membrane-altering toxins has been debated by many authors. Gaumann (1958) concluded that fusaric acid causes wilting of tomato plants by altering semipermeability of host cells resulting in a loss of solutes and a consequent loss of turgor. However, Duniway (1971) found no decrease in solute concentration or turgidity in infected tomato plants compared to healthy plants given the same water potential (above -12 bars). Additionally, transpiration rates of diseased plants were lower than in healthy plants. Furthermore, stem conductance of diseased plants was several times lower than in healthy plants. Duniway, therefore, concluded that wilting of tomato plants by Fusarium oxysporium f. sp. lycopersici is caused by a high xylem resistance to water flow.

Leaves of tomato plants treated with toxin produced by Corynebacterium michiganense wilted in advance of the toxin movement. Because vascular transport, as monitored by acid fuchsin movement, was not reduced, Rai and Strobel (1969) concluded that the toxin did not operate by occluding xylem vessels. Instead, they suggested that toxin disorganizes parenchymatous cell membranes, thereby resulting in a water imbalance. Strobel and Hess (1968) reported C. sepedonicum caused stem flaccidity before leaf wilting, a pattern that is not associated with vascular occlusion. Furthermore, water movement through potato was unaffected as evidenced by dye movement. Evidence for altered cell permeability included inability of cells to plasmolyze, electrolyte leakage, and electron micrographs showing membrane disruption. Daly (1981), however, refuted these studies claiming that inappropriate methods, including lack of replication and inappropriate choice of cell type for osmoregulation studies, were used and that data were insufficient to support the conclusions. In contrast, Daly supported the work of Wallis (1977) who suggested that vascular dysfunction in C. michiganense was the result of vascular cell wall degradation by pathogen-produced exoenzymes.

Stomatal effects

A different mechanism of water loss is illustrated by fusicoccin, a toxin produced by Fusicoccum amygdali, the pathogen causing canker of peach and almond. With this disease, wilting occurs in advance of fungal growth and host production of gums (Turner and Graniti 1969).

Fusicoccin stimulates potassium ion uptake by guard cells (Turner 1972), thereby increasing stomatal opening such that subsequent transpirational demand cannot be adequately satisfied.

Photosynthetic effects

Vascular diseases may also affect photosynthesis, either directly or indirectly. Any disease that causes leaf chlorosis or necrosis, as do many vascular diseases, can cause a photosynthetic reduction through disruption of chloroplasts and loss of chorophyll (Goodman et al. 1986). Additionally, stomatal closure in response to disease-caused water stress can reduce photosynthesis by decreasing CO_2. However, stomatal closure may occur independently of water stress, as happens with Fusarium wilt of tomato caused by F. oxysporum f. sp. lycopersici (Duniway and Slatyer 1971). Conversely, a disease that increases stomatal aperture may increase photosynthesis. A nonvascular-disease example of this is late blight of potatoes in which stomate opening is induced by infection with Phytophthora infestans (Farrell 1971). It is theoretically possible for a similar increase in photosynthesis to occur in a vascular disease in which stomatal opening is toxin-mediated.

Physiology of Brown Stem Rot-Infected Plants

Stem conductance

Few studies have been conducted regarding the physiology of brown stem rot-infected plants. Chamberlain and McAlister (1954) found that stem conductance was inversely proportional to disease severity. They reported a mean reduction in stem conductance of 50% for diseased stems, and speculated that the subsequent water stress and premature ripening resulted in reduced seed size on upper nodes, thereby reducing overall yield. Extracts made from diseased stems reduced stem conductance to 10-34% of normal (Chamberlain 1961). Activity of the extracts was unaffected by autoclaving, sterile filtering, or 1:5 dilution, and was only slightly reduced by 1:10 dilution. Reduction in water flow by the stem extract was irreversible. Because the extract caused internal browning, but no leaf wilting, Chamberlain concluded that the extract was not a toxin. He further concluded that reduction in water flow as measured by Chamberlain and McAlister (1954) was caused by a metabolite and not by physical plugging by mycelium. Further research by Gray and Chamberlain (1975) indicated that extracts made from plants infected with the Type I strain of *P. gregata* caused leaf wilting as well as internal browning. Extracts made from plants infected with the Type II strain caused vascular browning, but no wilting. This evidence lead them to conclude that Type I strains produce a leaf wilting toxin. Based on a dilution-series test, Gray and Chamberlain found the extract was effective at a 1:3 dilution, but not at a 1:9 dilution. Additionally, leaves of two resistant soybean

lines, PI 84.946-2 and PI 86.150, were unaffected by the extract. After following fast-green stain uptake through extract-treated leaves, they found that extracts from plants infected with either Type I or Type II strains reduced the rate of vascular transport, but did not prevent it. From the results of this experiment, combined with the differential symptomology produced by the two P. gregata isolates, they concluded that wilting was not caused entirely by plugging of vessels. They further concluded that the Type I strain produces a toxin which is responsible for the severe symptoms produced by the Type I isolate.

Gregatins

Five metabolites were isolated and identified by Kobayashi and Ui (1977) from cultures of P. gregata; they were named gregatin A, B, C, D, and E. These structurally similar metabolites are derivatives of tetronic acid. Although the gregatins were originally isolated from an adzuki bean isolate of P. gregata, Kobayashi and Ui (1980b) isolated gregatin A, C, and D from a soybean isolate of P. gregata from the U.S. Gregatin productivity by P. gregata, measured as mg gregatins produced from 100 ml culture filtrate, was comparable in an adzuki bean isolate from Japan and a soybean isolate of P. gregata from Iowa (Kobayashi and Ui 1980a); no mention was made of individual gregatins or their respective levels of production. Therefore, it is unclear whether or not soybean isolates of P. gregata produce gregatins B and E. Furthermore, gregatins have not been isolated from soybeans infected with P. gregata.

Studies of the phytotoxic effects of gregatins A, B, C, D, and E using cut tops from adzuki bean, mung bean, soybean, and kidney bean seedlings indicated that gregatins A, C, and D caused foliar symptoms in adzuki bean and mung bean at 50-100 ug/ml and more mild symptoms in soybean and kidney bean at 200 ug/ml (Kobayashi and Ui 1977). Gregatins B and E caused typical leaf symptoms in adzuki bean and mung bean and more mild symptoms in soybean and kidney bean at 200 ug/ml. Gregatins A, C, and D caused vascular browning in adzuki bean and mung bean at 50-100 ug/ml, but no vascular browning occurred in soybean and kidney bean even at 200 ug/ml. Gregatins B and E at 200 ug/ml caused no vascular symptoms in any of the plant species tested in this study.

Antibiosis

When tested for in vitro antimicrobial activity, the gregatins inhibited spore germination and mycelial growth of a wide range of fungi (Kobayashi and Ui 1977). Gregatin A was the most potent inhibitor. Gregatin C and D were comparable to each other in activity, but less active than gregatin A. Gregatin B and E also were comparable to each other, but less active than gregatin C and D. Gregatin A, C, and D were also inhibitory to the growth of several bacteria and a yeast while gregatins B and E were less inhibitory.

It has been suggested that graminin A, produced by Cephalosporium gramineum, may enhance survival of that pathogen by suppressing contaminants (Bruehl et al. 1969); the gregatins, produced by P. gregata, may perform a similar role (Kobayashi and Ui 1977). However,

no relationship between wheat straw decomposition and virulence or antibiotic-producing ability of Cephalosporium graminium was found (Mathre and Johnston 1979). Additionally, attempts to isolate gregatins from P. gregata-inoculated adzuki bean plants or from gregatin-injected plants were unsuccessful, indicating that either the isolation methodology was inappropriate or that the metabolite was structurally changed in planta (Mathre and Johnston 1979). Consequently, in vitro activities of gregatins cannot be applied directly to an in vivo system.

Photosynthesis

Reeder et al. (1986) investigated the role of compounds produced by P. gregata in inhibition of photosynthetic electron transport (PET). Of three extracts made from P. gregata cultures, they found that the mother liquor from gregatin A crystallization was the most potent inhibitor of PET, purified gregatin A was the least potent, and a crude extract was intermediate in potency. They concluded that two sites of inhibition exist. The sites are the photosystem II complex and the cytochrome b/f complex. They further suggested that inhibition of PET could cause the foliar symptoms that are sometimes caused by BSR.

SECTION I. EFFECT OF BROWN STEM ROT ON WATER RELATIONS AND PHOTOSYNTHESIS OF SOYBEAN

ABSTRACT

Phialophora gregata, causal agent of brown stem rot of soybean and adzuki bean, causes internal stem browning and interveinal chlorosis and necrosis of leaves. Water relations and photosynthesis were studied in diseased and healthy soybeans to determine the physiological effects of brown stem rot. A susceptible (Pride B216) and a resistant (BSR 201) cultivar were grown in the greenhouse and growth chamber and injection-inoculated at vegetative stage 1. A significant amount of disease developed in both cultivars, but Pride B216 was more highly stressed by disease than BSR 201. Stem conductance was reduced, and stomatal conductance was increased in both inoculated cultivars compared to uninoculated plants. Transpiration was increased more in Pride B216 than in BSR 201, and photosynthesis was significantly increased in BSR 201 plants when inoculated. Disease-caused water stress can be attributed to a combination of reduced stem conductance and increased water loss resulting from increased stomatal conductance. Additionally, results indicate that photosynthesis may be directly affected by disease rather than by disruption of stomatal control. Preliminary data indicating tolerance to disease by a resistant cultivar, exclusive of pathogen inhibition, are presented.

INTRODUCTION

Brown stem rot (BSR), caused by Phialophora gregata (Allington and Chamberlain) Gams, is a vascular disease of soybean and adzuki bean. Two isolate types of P. gregata have been identified (Gray 1971). The Type I isolate causes vascular browning as well as interveinal chlorosis and necrosis of leaves. The Type II isolate causes vascular browning but no foliar symptoms.

Chamberlain and McAlister (1954) reported that stem conductance is inversely proportional to the degree of internal browning and may be reduced to 50% of normal by BSR. Extracts made from both Type I- and Type II-infected soybean stems also can reduce stem conductance (Chamberlain 1961, Gray and Chamberlain 1975). Several metabolites (gregatins) have been isolated from cultures of P. gregata and have been found to cause both stem browning and leaf chlorosis in adzuki bean (Kobayashi and Ui 1977). These reports provide evidence that one or more metabolites of P. gregata may cause obstruction of xylem vessels, thereby reducing stem conductance. Such an effect may contribute to water deficiency in soybean, but it has been suggested (Gray and Chamberlain 1975) that wilting is not due merely to xylem occlusion.

Gray and Chamberlain (1975) suggested that a toxin is produced by the Type I isolate of P. gregata which is responsible for the foliar symptoms of BSR, but no speculation was made on a mechanism for such an effect. Reeder et al. (1986) examined the photosynthetic electron transport (PET)-inhibiting ability of three extracts made from cultures

of P. gregata. The mother liquor of gregatin A crystallization was the most potent inhibitor of PET, purified gregatin A was the least potent, and a crude extract was intermediate in potency. Because inhibition of PET can cause leaf chlorosis and necrosis, Reeder et al. (1986) propose that the leaf symptoms of BSR result from inhibition of PET by a P. gregata-produced metabolite.

Other measures of water status in P. gregata-infected soybean plants, such as stomatal conductance, transpiration, or leaf water potential, have not been examined. Additionally, no in vivo measurements of photosynthesis have been reported. The primary objective of this study was to characterize the water relations and photosynthesis of soybean as influenced by BSR. Second, physiological responses of a susceptible and resistant cultivar were compared.

METHODS AND MATERIALS

A resistant (BSR 201) and a susceptible (Pride B216) soybean cultivar were included in the study to provide an estimate of the extremes of plant response to brown stem rot. Pride B216 is the high yielding parent of BSR 201, and both cultivars are of maturity group II. Plants were grown in the greenhouse or a Conviron growth chamber. Greenhouse temperatures were maintained at about 24 C. The Conviron chamber was programmed for temperature to gradually increase from 18 C to 27 C in the morning and similarly decrease in the afternoon. Seeds were sown into 3.78-liter plastic pots containing a mixture of soil, peat, and sand (1:1:1). Approximately 5 g of Osmocote slow release fertilizer (14:14:14 N:P:K) were incorporated into each pot of soil mixture. Plants were watered as needed (usually twice daily) to maintain good growth.

Inoculum was produced using a modification of Mengistu and Grau's (1986) method. One hundred twenty-eight g green bean baby food (Gerber) was diluted with 500 ml distilled water, dispensed into three 500-ml Erlenmeyer flasks, and autoclaved for 25 min. The flasks of liquid medium were seeded with plugs of a Type I isolate of *P. gregata* grown on potato dextrose agar, and incubated two weeks at 21 C on a rotary shaker at 70 rpm. Inoculum was standardized to 10^7 spores/ml and divided into two samples. One sample was heated until boiling and allowed to cool to room temperature. A small amount of this sample was plated on potato dextrose agar to ensure the spores were nonviable. This spore suspension was used as an inoculum control. Plants were

injected at growth stage V1 (Fehr et al. 1971) below the cotyledon with 0.1 ml of either inoculum or boiled inoculum (control) using a hypodermic syringe and 23 gauge needle. Four trials of this experiment were conducted, and each cultivar X inoculation treatment was replicated at least 10 times.

Throughout the growing period, photosynthesis, internal CO_2, stomatal conductance, and transpiration were measured on the uppermost fully expanded leaf of each plant. Photosynthesis and internal CO_2 were measured in two of four experiments, with other parameters measured in all trials. Photosynthesis and internal CO_2 data were obtained using a Li-Cor Model LI-6200 photosynthesis meter. The abaxial and adaxial stomatal conductances and transpiration were evaluated with a Li-Cor Model LI-1600 steady state porometer, and whole leaf values were calculated from abaxial and adaxial values. Because they are destructive measurements, leaf water potential and stem conductance were obtained at the end of the experiments when the plants had reached the R6 stage. A PMS pressure chamber was modified and used to measure leaf water potential and stem conductance. Stem conductance was determined using a modification of Van Alfen and Turner's (1975b) technique. Each plant was cut off below the cotyledonary node, and the base of the stem was immersed in water and cut above the cotyledonary node to prevent entrapment of air bubbles. A 15 cm segment cut from the base of the stem was sealed into the pressure chamber with the base protruding into a glass centrifuge tube filled with degassed, distilled water inside the chamber. A small diameter tube was attached to the

top end of the stem segment leading to a 10 ml graduated cylinder. Five bars of pressure were applied for 30 sec., and the amount of water that collected in the graduated cylinder was recorded.

Disease severity was determined by splitting the stem, measuring the length of browning, and dividing that value by the height of the plant to calculate percentage stem browned (Tachibana and Card 1979). Additionally, isolations were made from each stem to determine the extent of P. gregata colonization. Sub-epidermal tissue was excised at each node and incubated for 10 days on potato dextrose agar modified with 135 mg/l streptomycin sulfate.

RESULTS AND DISCUSSION

Differences in physiological parameters were statistically insignificant until well into the disease development when plants were at pod fill stage; therefore, only data taken at the R6 stage are presented. Data from the four trials were combined to reduce variability, and are presented in Table 1. For many of the parameters measured, Pride B216 and BSR 201 responded differently to disease. Cultivar responses to BSR, expressed as percent of control, were determined for stem conductance, stomatal conductance, transpiration, photosynthesis, and leaf water potential (Fig. 1).

Approximately 0.8% stem browning occurred in uninoculated plants in tissues contiguous to the site of boiled inoculum injection and may have resulted from mechanical injury, plant responses to dead spores, or limited saprophytic activity by contaminants at the injury site (Table 1). Attempts to isolate P. gregata from control plants were, in all cases, unsuccessful (Table 2). Therefore, plants inoculated with nonviable spore suspensions were verified as controls. Isolation of P. gregata from inoculated plants was achieved readily, indicating that infection had occurred.

Significantly more internal stem browning occurred in both inoculated Pride B216 (54%) and BSR 201 (42%) plants than the corresponding control plants (Table 1). Furthermore, among inoculated plants, disease severity was significantly higher in Pride B216 than in BSR 201 plants. In trial four, both browning and P. gregata colonization were determined. As in the other experiments, browning in

Table 1. Carbon exchange rates (CER), internal CO_2 (Cint), transpiration rates (Trans), stomatal conductance (Stom), leaf water potential (LWP), stem conductance (Stem), and percent stem browning (BSR) of two soybean cultivars as affected by P. gregata

		Physiological and Disease Parameters[a]						
Cultivar	Inoc.[b]	CER $umol/m^2/s$	Cint ppm	Trans $mmol/m^2/s$	Stom cm/s	LWP bar	Stem ml/30 s	BSR[c] %
Pride B216	I	5.22	302.3	3.4	173	-10.9	3.0	54.3
	C	4.72	320.2	2.5	123	-9.8	5.5	0.8
BSR 201	I	4.63	318.8	3.0	153	-9.6	3.3	41.8
	C	2.46	315.2	2.4	122	-10.0	4.0	0.8
LSD (P=0.05)		2.02	NS	0.7	27	-1.0	0.6	7.5

[a]Based on combined data from 10-18 replicates per experiment and four experiments.

[b]Inoc.=I (plants injected with viable spore suspension) or C (plants injected with nonviable spore suspension).

[c]BSR=percentage of stem length browned.

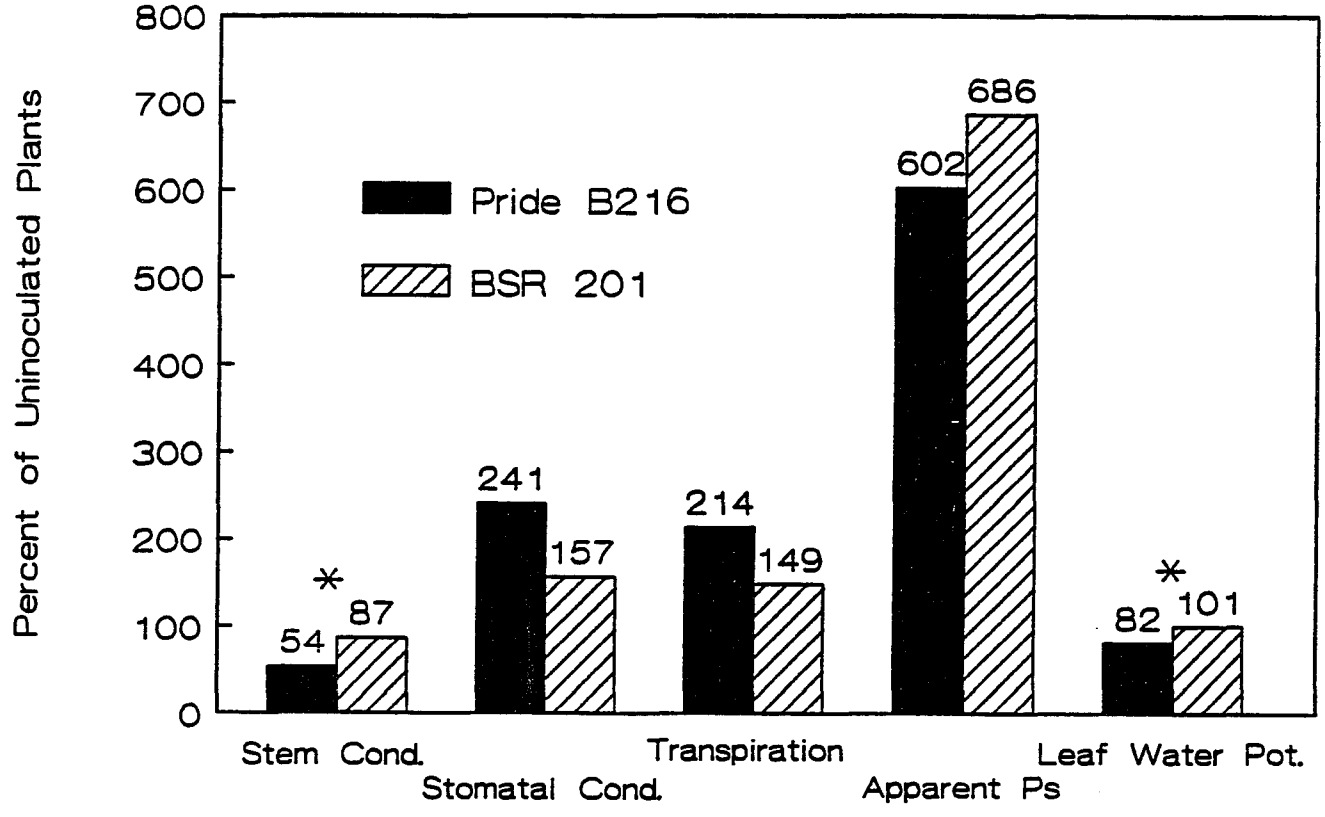

Figure 1. Stem conductance, stomatal conductance, transpiration, apparent photosynthesis, and leaf water potential responses of inoculated Pride B216 and BSR 201 plants expressed as percent of uninoculated plants. Values were determined by dividing measurements from inoculated plants by measurements from uninoculated plants for each replication X treatment combination prior to statistical analysis. Bars sharing * are significantly different by studentized T-test (P=0.05)

Table 2. Percent stem browning and P. gregata colonization in plants of two soybean cultivars inoculated with a suspension of viable (I) or nonviable (C) P. gregata spores

Cultivar	Inoculation treatment[a]	Internal browning[b]	P. gregata colonization[b]
Pride B216	I	16.8	48.1
	C	0	0
BSR 201	I	4.1	7.1
	C	0	0
LSD (P=0.05)		4.3	7.5

[a]I=plants injected with viable spore suspension; C=plants injected with boiled spore suspension.
[b]Based on 10 replications.

trial four occurred to a significantly greater extent in inoculated Pride B216 (17%) than BSR 201 plants (4%). Moreover, Pride B216 plants were colonized by P. gregata to a greater extent than BSR 201 plants. Stem colonization averaged 48% for Pride B216 and 7% for BSR 201 (Table 2).

Stem conductances were 5.5 and 4.0 ml/30 sec for controls of Pride B216 and BSR 201, respectively, and were reduced to 3.0 and 3.3 ml/30 sec in diseased Pride B216 and BSR 201, respectively (Table 1). Disease caused an overall reduction of 34% in stem conductance. This finding is in proportion to the nearly 50% loss of conductance reported by Chamberlain and McAlister (1954) with the discrepancy between studies probably resulting from differences in cultivars and

methodologies. Chamberlain and McAlister used a 7 cm stem segment, and applied 15 psi (one bar) pressure for four min., whereas we used a 15 cm segment, and applied five bars pressure for 0.5 min. Additionally, disease severity cannot be directly compared between the two studies because Chamberlain and McAlister measured the degree (light to heavy) of browning at basal cross-sections rather than extent of internal stem browning. Stem conductances in inoculated Pride B216 and BSR 201 plants were 54% and 87% of control, respectively (Figure 1). These percentage values were significantly different at the P=0.05 level by T-test.

Because stem conductance was reduced in inoculated plants, decrease in stomatal conductance and transpiration was expected, characteristic with other vascular diseases (Threlfall 1959, Van Alfen and Turner 1975a,b). However, stomatal conductance was higher, 173 and 153 cm/sec, in inoculated Pride B216 and BSR 201 plants, respectively, in contrast to 123 and 122 cm/sec in control plants (Table 1). Although the differences were statistically significant for both cultivars, the cultivar responses to disease (expressed as percent of control) were not statistically different from each other (Figure 1). Increased stomatal conductance suggests that a hormone or toxin was disrupting stomatal regulation. Because P. gregata is known to produce metabolites that have produced disease symptoms in in vitro systems (Gray and Chamberlain 1975, Kobayashi and Ui 1977), it seems likely that one or more of these metabolites might affect stomatal control. A similar disease response occurs with canker of peach and almond, caused

by Fusicoccum amygdali (Turner and Graniti 1969). F. amygdali produces a toxin, fusicoccin, that stimulates K^+ uptake by guard cells (Turner 1972). Consequently, stomatal apertures are increased to the extent that the resulting transpirational demand cannot be adequately met.

The increased stomatal conductance, resulting from soybean inoculation with P. gregata, caused a corresponding increase in transpiration for both cultivars (Table 1), similar to the response of almond and peach plants to F. amygdali. However, only the increase from 2.5 to 3.4 mmol/m^2/sec for Pride B216 was statistically significant. The increase from 2.4 to 3.0 mmol/m^2/sec for BSR 201 was not statistically significant because of high variability among replicates. As with stomatal conductance, the transpiration response between cultivars was not statistically significant (Figure 2).

A significant increase from 2.46 to 4.63 umol/m^2/sec in photosynthesis occurred in inoculated BSR 201 plants (Table 1). A smaller, nonsignificant increase from 4.72 to 5.22 umol/m^2/sec in photosynthesis was observed in Pride B216 plants. The increase in photosynthesis could be, in part, a consequence of increased stomatal conductance which would have allowed a greater rate of CO_2 uptake. To examine this possibility, I calculated partial correlation coefficients for all variables and photosynthesis. The partial correlation coefficient between photosynthesis and stomatal conductance was R=0.36, significant at P=0.05 yet quite low, indicating that photosynthesis was controlled by more than stomatal conductance. Internal CO_2 values were not significantly affected by inoculation treatment (Table 1).

However, Increased photosynthesis in inoculated plants may have masked the increased CO_2 concentrations expected with increased stomatal conductance. Reeder et al. (1986) reported that a metabolite of P. gregata inhibits PET in vitro and suggested that a similar in vivo inhibition could cause leaf chlorosis and necrosis. Gray and Chamberlain (1975) reported that two BSR-resistant soybean plant introductions were resistant to wilting induced by extracts from P. gregata-infected plants. The BSR-resistant plants could be resistant to the activity of a metabolite produced by P. gregata. It is possible that the potential increase in photosynthesis caused by increased stomatal conductance is inhibited by a P. gregata metabolite in susceptible soybean cultivars, but not in resistant cultivars. Although a greater apparent photosynthesis response for BSR 201 than Pride B216 was obtained (Figure 1), the cultivar responses were not significantly different by Studentized T-test (P=0.05).

Leaf water potential provides an index of plant water status; low leaf water potential indicates disruption of water relations. BSR significantly reduced leaf water potential in Pride B216 but not BSR 201, although a significant amount of internal browning occurred in both cultivars (Table 1, Figure 1). As mentioned previously, percent reduction in stem conductance was also greater for Pride B216 than for BSR 201 (Figure 1). These results indicate that BSR caused greater water stress in Pride B216 than in BSR 201.

Data from this study suggest that water relations in P. gregata-infected soybean plants are disrupted by a combination of reduced stem

conductance and increased water loss resulting from increased stomatal conductance. Photosynthesis may also be affected, but whether directly or indirectly remains unclear. These effects may be attributed to a metabolite produced by P. gregata. However, additional work is needed to confirm this hypothesis. The mechanism by which stem conductance is reduced must be elucidated. Van Alfen and Turner (1975a,b) reported that toxins occluded xylem vessels by virtue of their large molecular weight. A similar mechanism of stem conductance reduction with BSR is unlikely due to the low molecular weight of gregatins. Future study needs to focus on stomatal control as affected by extracts made from P. gregata culture filtrates and from diseased soybean stems. The effect upon cuticular transpiration should be evaluated, and the relative importance of reduced stem conductance and increased stomatal conductance needs to be determined. P. gregata metabolites have been implicated in reduction of stem conductance, enhancement of stomatal conductance, and inhibition of PET. Although several secondary metabolites have been isolated (Kobayashi and Ui 1977), the compounds responsible for the plant responses have not been determined. The number and specific involvement of secondary metabolites in pathogenesis remains to be investigated.

Although extent of stem browning provides a relative measure of disease severity, it does not represent the limits of colonization. Colonization may extend well beyond stem browning in the susceptible cultivar (Table 2). No general conclusions regarding mechanism of resistance can be made based on only two cultivars; however, a

preliminary insight is provided. Given a significant amount of internal browning, resistant and susceptible cultivars may vary in their degree of physiological response. For example, stem conductance and leaf water potential were less affected in BSR 201 than Pride B216 although over 40% internal browning occurred in the resistant cultivar (Table 1). Thus, studies evaluating the responses of an array of resistant and susceptible cultivars are needed to determine the validity of this phenomenon. Assuming that metabolites are significantly involved in pathogenicity, it is possible that more metabolites are produced in susceptible cultivars due to more extensive colonization or that resistant cultivars inhibit metabolite production and activity as well as pathogen growth.

SECTION II. GROWTH AND YIELD ANALYSIS OF SOYBEAN AS AFFECTED BY BROWN STEM ROT

ABSTRACT

Brown stem rot (BSR), caused by Phialophora gregata, is a vascular disease of soybean. Its effects on soybean growth and yield were evaluated using a resistant (BSR 201) and a susceptible (Pride B216) cultivar. Plants were greenhouse-grown and inoculated with viable or nonviable P. gregata spore suspensions. Disease significantly reduced leaf area, branching, number of nodes and leaves, number and weight of pods, number and weight of seeds per plant, number of full-seeded pods, and number of seeds per pod in both cultivars. Single seed weight of the susceptible cultivar, Pride B216 was significantly reduced by BSR in one experiment. Yield reduction resulted both from reduction in seed number and in seed size. It is suggested that disruption of water relations by BSR reduces yield by limiting photosynthetic leaf area and by increasing seed abortion. Diseased BSR 201 plants tolerated leaf area loss and sustained pod fill better than Pride B216.

INTRODUCTION

Phialophora gregata (Allington and Chamberlain) Gams causes brown stem rot (BSR) of soybean [Glycine max (L.) Merr], a systemic disease characterized by vascular browning and interveinal leaf chlorosis and necrosis. In fields where BSR occurs, yield losses of 5-56% have been reported (Dunleavy and Weber 1967, Gray 1972a, Gray and Sinclair 1973, Kennedy and Lambert 1981, Mengistu and Grau 1987, Mengistu et al. 1987, Weber et al. 1966). Variation in loss estimates is due to several factors including inoculation method, isolate type, cultivar resistance or susceptibility, cropping history, and environment. BSR is becoming more prevalent in US soybean production areas (Tachibana and Booth 1979), consequently, yield losses from this disease continue to increase. Until about 1981, crop rotation with nonsusceptible crops for at least three years was the only control available (Dunleavy and Weber 1967). Several BSR resistant lines have been released recently (Tachibana 1986). Current culture practices include planting of soybean in alternate years, an inadequate rotation for BSR control. Therefore, resistant cultivars provide the most feasible control strategy.

BSR has caused early maturity (Gray 1972a, Tachibana 1982, Weber et al. 1966). Speculation that yield loss may be due to premature ripening has been suggested (Chamberlain and McAlister 1954). However, yield losses without maturity effects have been documented. Kennedy and Lampert (1981) reported a yield reduction of 13% under continuous soybean culture compared to rotation culture, with little difference in

maturity between treatments. Similarly, Weber et al. (1966) found that soybeans grown on P. gregata-infested land were 2.5 cm taller, matured only two days earlier, and were 8% less susceptible to lodging than soybeans grown on noninfested land. Furthermore, yield was 11% less, plants produced 10.4% fewer seeds, and 100 seed weight was 0.1 g less from infested land than from noninfested land. Weber et al. (1966) calculated that 94% of BSR yield loss was due to seed number reduction and 6% was due to seed size reduction. In comparison, Dunleavy and Weber (1967) reported that 64% of the yield losses associated with continuous soybean culture (severe BSR conditions) resulted from a reduction in seed number and 36% of the losses were due to a reduction in seed size. Specifically, they found that seed size was reduced 2 g per 100 seeds and seed number was reduced 28%.

Few studies compare yield characteristics of BSR diseased and healthy soybeans. Mengistu et al. (1986) found a 17% yield advantage with resistant cultivars over susceptible cultivars, and reported that yield was influenced more by the number of seed-bearing pods than by seed size.

Although limited yield analyses have been conducted, analyses of growth parameters affected by BSR have not been reported. This information is necessary to identify probable sources of yield reduction. The primary objective of this study was to analyze both growth and yield parameters of soybean as affected by BSR and to relate these parameters to each other. Secondarily, the study made use of the

growth and yield analyses to evaluate the potential yield advantage of a resistant cultivar over a susceptible cultivar.

METHODS AND MATERIALS

Soybean plants of a susceptible (Pride B216) and a resistant (BSR 201) cultivar (both are maturity group II and Pride B216 is the high yielding parent of BSR 201) were grown in the greenhouse in 3.78-liter plastic pots filled with a sand:soil:peat (1:1:1) mixture. Five grams Osmocote slow release fertilizer (14:14:14) was incorporated into the soil of each pot. Pots were watered twice daily, and rooms were maintained at about 24 C.

Inoculum was produced in 500-ml flasks by seeding 250 ml of a sterile, green bean medium (128 g Gerber baby food green bean in 500 ml distilled water) with plugs of P. gregata grown on potato dextrose agar. The broth cultures were incubated two weeks at 21 C on a rotary shaker at 70 rpm (Mengistu and Grau 1986). Spores were collected by vacuum filtration and spore suspensions were adjusted to 10^7 spores/ml. Half of the volume was heated to boiling to kill the spores. This nonviable spore suspension served as the inoculum control. Plants were inoculated at the V1 stage (Fehr et al. 1971) with either a viable or nonviable P. gregata spore suspension. An aliquot of 0.1 ml of either viable inoculum or boiled spore suspension (control) was injected into each hypocotyl using a sterile syringe and 23 gauge needle. A Type I isolate of P. gregata was used throughout the study.

Several times during plant development, measurements were made for leaf area per plant, plant height, vegetative and reproductive stages, and number of branches, nodes, leaves, and pods. At the termination of the experiment, stem, leaf, root, pod, and seed dry weights were

measured. Prior to threshing, pods were categorized as one-, two-, three-, or four-seeded, or immature (less than R5). Pods of R5 or higher were counted to determine the number of full-seeded pods. Number of seeds per plant was calculated from the pod data, and average single seed weight was determined by dividing seed weight per plant by seed number per plant. Root:shoot ratios were calculated from dry weight values. Disease severity was evaluated by splitting stems and measuring the extent of internal browning relative to plant height (Tachibana and Card 1979).

Three experiments (I-III) were conducted, and for all experiments each treatment was replicated at least 10 times. Experiment I was performed from October through January, experiment II from November through February, and experiment III from December through March.

RESULTS AND DISCUSSION

Inoculated plants had good disease symptom development, and only a trace of vascular or pith browning occurred in control plants, possibly a reaction of the plants to killed spores (Table 1). The susceptible cultivar, Pride B216 showed a higher disease severity than the resistant cultivar, BSR 201, in experiments I and II.

In experiment I, leaf area, branching, number of nodes and leaves, and number of pods were reduced during the initial readings at reproductive stage 2.5 (R2.5) in diseased plants of both Pride B216 and BSR 201 when compared with uninoculated controls (Table 2). A height reduction by BSR was observed in Pride B216 by R4.7. These effects were evident until termination of the experiment. In experiment II, the same effects were seen by R2.3 in Pride B216 but not in BSR 201 (Table 3), possibly because of the lower disease severity (Table 1); however, the effects were no longer evident by R4.4. At termination of experiment I, pod weight, seed weight per plant, number of full-seeded pods, and number of seeds per plant were significantly reduced by disease in both cultivars (Table 4). Single seed weight was reduced by BSR in Pride B216 and number of seeds per pod was reduced in BSR 201. In experiment II, pod weight, seed weight per plant, and number of seeds per pod were lower due to disease only in Pride B216 (Table 5); BSR 201 was unaffected by inoculation (Table 5) although some disease developed (Table 1). No significant growth or yield effects due to BSR were seen in experiment III (data not shown), although significant correlations between parameters occurred (Table 8) and disease was

evident by harvest (Table 1).

In experiment I, reduction in total seed weight per plant by BSR was accompanied by a reduction in the number of seeds per plant for both cultivars, and in the single seed weight only for Pride B216, (Table 4). In experiment II Pride B216 had a similar trend in seed weight reduction because of BSR (Table 5). Inoculation had no effect on the number of seeds per plant nor the single seed weight in experiment II; the trends, however, were similar to those of experiment I. The number of seeds per pod was reduced by disease in Pride B216. Therefore, yield reduction from BSR was influenced by seed number and, to a lesser extent, by seed size. The conclusion is further supported by partial correlation analysis. There was no significant correlation between disease severity and single seed weight in any of the three experiments (Tables 6, 7, 8). However, there was a significant negative correlation between disease severity and seed weight per plant in experiments I and III (Table 6 and 8). These data suggest that BSR had a greater effect on seed number than on seed size. However, under conditions of intense disease pressure, yield reduction was also influenced by seed size as was seen in experiment I. The influence of single seed weight on yield (seed weight per plant) was also indicated by correlation values (Tables 6, 7, 8).

A reduction in pod number due to BSR was evident throughout experiment I (Table 2) and was reflected also in both the final pod weight and seed weight per plant (Table 4). However, although a reduction in pod weight and total seed weight per plant occurred in

Pride B216 in experiment II (Table 5), a similar reduction in pod number was not evident at R4.4 (Table 3). These data indicate that yield reduction associated with BSR resulted partly from reduction in pod number, but to a greater extent from the reduced number of seeds produced per pod.

In experiments I and II branching correlated significantly with leaf area as well as numbers of pods and seeds produced per plant (Tables 6, 7). These results suggest that branching influenced seed number by providing additional reproductive sites as well as photosynthetic leaf area. Because more leaf area was available in the noninoculated plants, there was a greater photosynthetic base from which to fill pods. Indeed, the number of full-seeded pods was significantly reduced by disease in both cultivars in experiment I (Table 2). Therefore, leaf area probably had a significant effect on pod filling. In fact, there was a significant correlation between leaf area and seed number as well as number of full-seeded pods (Tables 6, 7, 8).

Yield (seed weight per plant) was reduced by inoculation in Pride B216 by 65% in experiment I and by 20% in experiment II, and in BSR 201 by 48% in experiment I and by 0% in experiment II. With comparable leaf area reductions by BSR (ca. 51%) between cultivars in experiment I, there was a greater percentage reduction in number of full-seeded pods due to inoculation for Pride B216 (47%) than for BSR 201 (34%) and in seed weight per plant for Pride B216 (65%) than for BSR 201 (47%). Such effects imply that the resistant plants tolerated leaf area loss

better than did susceptible plants. Further studies comparing additional resistant and susceptible cultivars are needed to determine whether this trend is common to all resistant cultivars.

Muchow et al. (1986) studied the effects of water deficits on field-grown soybeans using the cultivar Biloxi. None of the plants in that experiment produced branches, but a water deficit caused a reduction in both leaf area and number of nodes produced. A mild water deficit caused a 12% reduction in leaf area and a 4-7% reduction in number of nodes. In our study, disease caused leaf area reductions of up to 53% and node reductions of up to 31%, implying that BSR induces a mild water deficit in soybean. Muchow et al. (1986) further found that water deficits, including mild ones, reduce stomatal conductance. Data from our lab (unpublished) indicate that BSR causes an increase in stomatal conductance. Hence, although similarities exist between plant responses to BSR and to water deficit, there are fundamental differences between the two stresses. This implies that interactions between water stress and BSR may be possible. Such an interactive effect was documented (Mengistu et al. 1987) when yield reduction by BSR was significantly greater under conditions of combined water stress and BSR than when these stresses occurred alone.

Our results confirmed the relative importance of seed number over seed size in yield reduction by BSR and indicated the importance of photosynthetic leaf area in accumulating yield under disease conditions. Boyer (1970) showed that leaf area responds early to water deficit, and Shibles et al. (1975) reported that seed abortion occurs

due to inadequate water. It follows, then, that disruption of water relations by BSR may reduce yield by limiting photosynthetic leaf area and by increasing seed abortion. Although observed effects on yield components can be explained by disease-induced water stress, these effects also could occur with altered hormone levels. Consequently, it is possible that the disease, in addition to influencing water relations, may have an effect on hormonal control. However, direct measurements of hormone levels are not available to test this possibility; therefore, it is unclear whether BSR does influence hormonal regulation.

Table 1. Percentage stem browning in Pride B216 and BSR 201 plants inoculated (I) with P. gregata or uninoculated (C) in three experiments

		Experiment		
Cultivar	I/C	I	II	III
Pride B216	I	83[a]	54	63
	C	0	0	4
BSR 201	I	66	37	65
	C	0	2	0
LSD (0.05)		14	7	17

[a]Percentage of stem length browned in pith or vascular tissue.

Table 2. Reproductive stage (Repro. stage), leaf area, height (Ht.), vegetative stage (Veg. Stage), and number (No.) of branches (Br.), nodes (Node), leaves (Lvs.), and pods (Pods) on Pride B216 and BSR 201 plants inoculated (I) with P. gregata or uninoculated (C): from experiment I with data collected at three stages

Cultivar	I/C	Repro. Stage[a]	Leaf Area[b]	Ht.[c]	Veg. Stage[a]	No. Br.	No. Node	No. Lvs.	No. Pods
Initial Readings									
Pride B216	I	2.2	884	94	10.5	0.5	13.9	13.2	2.5
	C	2.6	1237	80	10.9	2.0	17.8	17.8	8.1
BSR 201	I	2.5	744	66	8.9	0.4	11.5	11.3	5.4
	C	2.6	1266	73	9.4	2.1	17.1	17.1	10.8
LSD (0.05)		NS	259	NS	NS	1.1	3.2	3.3	3.0
Second Readings									
Pride B216	I	4.4	1361	165	16.5	1.5	24.0	19.2	20.6
	C	4.9	2199	135	16.3	5.0	34.7	33.9	36.4
BSR 201	I	4.7	1263	102	14.0	0.5	16.9	12.9	20.3
	C	4.9	2114	112	14.2	2.3	25.2	24.9	38.1
LSD (0.05)		NS	539	27	NS	1.3	5.8	5.6	8.8
Final Readings									
Pride B216	I	5.0	1009	164	16.6	1.5	25.2	18.3	23.3
	C	5.6	1943	135	16.5	5.1	34.9	32.4	36.3
BSR 201	I	5.5	984	102	14.4	0.5	17.4	12.8	22.8
	C	5.7	2086	111	14.1	2.3	25.4	24.7	33.3
LSD (0.05)		NS	500	29	NS	1.3	6.2	6.0	8.6

[a]As defined by Fehr et al. 1971.
[b]Leaf area of whole plant in cm^2.
[c]Height of plant in cm.

Table 3. Leaf area, height (Ht.), vegetative stage (Veg. Stage), reproductive stage (Repro. stage), and number (No.) of branches (Br.), nodes (Node), leaves (Lvs.), and pods (Pods) of Pride B216 and BSR 201 plants inoculated (I) with *P. gregata* or uninoculated (C); from experiment II with data collected at two stages

Cultivar	I/C	Repro. Stage[a]	Leaf Area[b]	Ht.[c]	Veg. Stage[a]	No. Br.	No. Node	No. Lvs.	No. Pods
Initial Readings									
Pride B216	I	2.3	1557	108	13.0	3.8	23.4	22.9	4.1
	C	2.5	1848	99	12.4	5.3	27.3	27.3	8.8
BSR 201	I	2.1	2136	106	13.3	2.4	22.1	22.1	5.6
	C	2.1	2077	98	12.4	2.3	21.6	21.6	6.9
LSD (0.05)		NS	281	9	0.7	1.1	3.3	3.3	4.0
Final Readings									
Pride B216	I	4.2	2406	149	15.5	6.4	42.1	36.6	53.3
	C	4.6	2658	142	14.7	6.6	42.0	38.0	57.9
BSR 201	I	4.3	2735	147	15.8	2.7	30.8	26.7	48.1
	C	4.4	2700	134	14.8	3.4	31.6	27.9	48.9
LSD (0.05)		NS	NS	NS	NS	NS	NS	NS	NS

[a]As defined by Fehr et al. 1971.
[b]Leaf area of whole plant in cm^2.
[c]Height of plant in cm.

Table 4. Stem, leaf, root, pod, and seed dry weights (Wt.), root:shoot ratio (R:S), number (No.) of seeds per pod (seed/pod), full-seeded pods, (Full Pods), and seeds per plant (Seed/Pl.), and single seed weight (Ssd. Wt.) of Pride B216 and BSR 201 plants inoculated (I) with P. gregata or uninoculated (C) from experiment I

Cultivar	I/C	Stem Wt. (g)	Leaf Wt. (g)	Root Wt. (g)	Pod Wt. (g)	Seed Wt. (g)	R:S	No. Seeds /Pod	No. Full Pods	No. Seed /Pl.	Ssd. Wt.
Pride B216	I	2.9	2.4	1.3	4.2	2.6	0.20	2.1	16.0	35.2	.095
	C	3.1	4.1	1.5	11.8	7.4	0.22	2.1	30.0	63.3	.130
BSR 201	I	2.1	2.2	0.7	5.7	4.2	0.20	1.9	19.2	36.5	.122
	C	2.7	4.0	1.4	11.4	8.0	0.21	2.2	28.9	62.1	.131
LSD (0.05)		NS	NS	NS	2.7	1.7	NS	0.3	6.6	14.2	.026

Table 5. Stem, leaf, root, pod, and seed dry weights (Wt.), root:shoot ratio (R:S), number (No.) of seeds per pod (seed/pod), full-seeded pods, (Full Pods), and seeds per plant (Seed/Pl.), and single seed weight (Ssd. Wt.) of Pride B216 and BSR 201 plants inoculated (I) with P. gregata or uninoculated (C) from experiment II

Cultivar	I/C	Stem Wt. (g)	Leaf Wt. (g)	Root Wt. (g)	Pod Wt. (g)	Seed Wt. (g)	R:S	No. Seeds /Pod	No. Full Pods	No. Seed /Pl.	Ssd. Wt.
Pride B216	I	5.7	7.8	2.1	12.9	7.3	0.16	2.0	41.2	81.1	.089
	C	5.3	8.6	2.6	15.5	9.1	0.24	2.1	43.9	91.9	.105
BSR 201	I	6.5	9.6	3.3	13.6	7.6	0.21	1.9	38.8	73.1	.105
	C	6.2	10.1	3.3	13.6	7.3	0.20	1.9	40.2	77.4	.091
LSD (0.05)		NS	NS	NS	2.2	1.7	NS	0.1	NS	NS	NS

Table 6. Pearson correlation coefficients of some growth and yield parameters and disease severity of soybean from experiment I

	Seeds[a]	Pods[b]	Leaf area[c]	Branches[d]	Leaves[e]	Full seeded pods[f]	Single seed weight[g]	Total seed wt[h]
BSR[i]	-.65***	-.49**	-.56***	-.55***	-.52**	-.64***	NS	-.67***
Seeds		.92***	.81***	.62***	.75***	.98***	-.33*	.60***
Pods			.75***	.57***	.71***	.95***	-.41*	.39*
Leaf area				.61***	.87***	.81***	-.34*	.33*
Branches					.89***	.58***	NS	.37*
Leaves						.72***	NS	NS
Full seeded pods							-.36*	.53***
Single seed weight								.46**

[a]Number of seeds per plant.
[b]Number of pods per plant.
[c]Leaf area per plant.
[d]Number of branches per plant.
[e]Number of leaves per plant.
[f]Number of full-seeded pods per plant.
[g]Single seed weight.
[h]Total seed weight per plant.
[i]Disease severity.
* Significant at the P=0.05 level.
** Significant at the P=0.005 level.
*** Significant at the P=0.0005 level.

Table 7. Pearson correlation coefficients of some growth and yield parameters and disease severity of soybean from experiment II

	Seeds[a]	Pods[b]	Leaf area[c]	Branches[d]	Leaves[e]	Full seeded pods[f]	Single seed weight[g]	Total seed wt[h]
BSR[i]	-.24*	NS	-.29*	NS	NS	NS	NS	NS
Seeds		.85***	.73***	.51***	.65***	.95***	NS	.58***
Pods			.66***	.45***	.61***	.79***	NS	.37***
Leaf area				.25*	.57***	.72***	NS	.29*
Branches					.78***	.41***	-.26*	NS
Leaves						.59***	-.41***	NS
Full seeded pods							NS	.57***
Single seed weight								.71***

[a]Number of seeds per plant.
[b]Number of pods per plant.
[c]Leaf area per plant.
[d]Number of branches per plant.
[e]Number of leaves per plant.
[f]Number of full-seeded pods per plant.
[g]Single seed weight.
[h]Total seed weight per plant.
[i]Disease severity.
* Significant at the P=0.05 level.
** Significant at the P=0.005 level.
*** Significant at the P=0.0005 level.

Table 8. Pearson correlation coefficients of some growth and yield parameters and disease severity of soybean from experiment III

	Seeds[a]	Pods[b]	Leaf area[c]	Branches[d]	Leaves[e]	Full seeded pods[f]	Single seed weight[g]	Total seed wt[h]
BSR[i]	NS	-.33*	NS	-.35*	-.37*	NS	NS	-.31*
Seeds		.81***	.77***	NS	.80***	.96***	-.70**	.42*
Pods			.92***	NS	.86***	.74***	-.59***	.31*
Leaf area				NS	.86***	.69***	-.45**	.44**
Branches					.51**	NS	NS	.55***
Leaves						.72***	-.51**	.43*
Full seeded pods							-.72***	.33*
Single seed weight								.33*

[a]Number of seeds per plant.
[b]Number of pods per plant.
[c]Leaf area per plant.
[d]Number of branches per plant.
[e]Number of leaves per plant.
[f]Number of full-seeded pods per plant.
[g]Single seed weight.
[h]Total seed weight per plant.
[i]Disease severity.
* Significant at the P=0.05 level.
** Significant at the P=0.005 level.
*** Significant at the P=0.0005 level.

SUMMARY

The overall objective of this study was to determine the effects of brown stem rot (BSR) on certain parameters of soybean growth and physiology. Specifically, the study identified growth and yield parameters affected by BSR and characterized the influence of this disease on water relations and photosynthesis of soybean. The study was conducted by comparisons between a resistant and a susceptible cultivar.

Soybean plants of a resistant cultivar, BSR 201, and a susceptible cultivar, Pride B216, were grown in the greenhouse and inoculated with spore suspensions of Phialophora gregata. Physiological and growth parameters were measured throughout plant development, and yield parameters were measured at the R5-6 stage. Physiological parameters included stomatal conductance, transpiration, leaf water potential, stem conductance, and apparent photosynthesis. Growth and yield parameters included leaf area per plant, plant height, vegetative and reproductive stages, number of branches, nodes, leaves, and pods, number of full-seeded pods, number of seeds per pod and per plant, and stem, leaf, root, pod, total seed, and single seed dry weights. Disease severity was measured as percentage stem browned.

Both inoculated BSR 201 and Pride B216 plants developed vascular and pith browning, but disease severity was greater in Pride B216. Although effects of disease on growth parameters were evident early in plant development, physiological differences between diseased and healthy plants were not measurable until late in plant and disease

development. Reduction in stem conductance and increase in stomatal conductance occurred in both inoculated BSR 201 and Pride B216 plants when compared with uninoculated controls. Transpiration was increased more in diseased Pride B216 than in BSR 201, and photosynthesis was significantly increased in diseased BSR 201, but not Pride B216, plants. BSR caused greater water stress in Pride B216 than in BSR 201. Disease-caused water stress was attributed to a combination of reduced stem conductance and increased water loss resulting from increased stomatal conductance.

In both cultivars, disease significantly reduced leaf area, branching, number of nodes and leaves, number and weight of pods and seeds per plant, number of full-seeded pods, and number of seeds per pod. Single seed weight was reduced by disease in Pride B216 in one experiment. Disease-associated yield loss resulted from reduction in seed number and, to a lesser extent, reduction in seed size.

BSR stressed soybeans by reducing stem conductance and increasing water loss through leaves. The results of this stress were reductions in leaf area and number of seeds produced, resulting in yield loss.

Given a significant amount of internal stem browning, resistant and susceptible cultivars may vary in their degree of physiological response to disease. Stem conductance and leaf water potential were less affected in inoculated BSR 201 than Pride B216 although over 40% internal browning occurred in the resistant cultivar. Under conditions of high disease severity and comparable leaf area reductions (ca. 51%) between cultivars, there was a greater percentage reduction in the

number of full-seeded pods and in seed weight per plant due to BSR for Pride B216 than for BSR 201. Thus, resistant plants may tolerate effects of disease better than susceptible plants. Further research is needed to confirm this hypothesis. Plant responses should be compared between resistant and susceptible plants with similar disease severities. Additionally, an array of resistant and susceptible cultivars must be examined to determine the range of this phenomenon. Furthermore, studies determining the role of P. gregata-produced metabolites are needed. If metabolites are involved in pathogenicity, resistance may operate by indirectly inhibiting metabolite production through pathogen inhibition or by inhibiting metabolite activity.

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